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TRENDS

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Capillary electrophoresis in pharmaceutical and biomedical analysis

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Since its introduction in the 1980s [1, 2], capillary electrophoresis (CE) has become established as an attractive and complementary alternative to chromatographic techniques such as liquid and gas chromatography. Indeed, since both CE and chromatographic techniques use different separation mechanisms, the methods can be considered as orthogonal.

Capillary electrophoresis has rapidly become a powerful separation technique for large and small molecules, which can be organic or inorganic, and has found several applications in different fields, such as environmental, clinical, forensic, biochemical and pharmaceutical analysis: its high efficiency (up to 1 million theoretical plates have been observed therefore allowing very difficult separations), short analysis time and method development, simple instrumentation, low sample and solvent consumption are the main reasons for this success. CE can be applied in aqueous and non-aqueous (NACE) media and can be coupled with several detectors [3]. Moreover, owing to the large efficiencies obtained in CE, this technique exhibits impressive results for the separation of enantiomers. For this purpose, a chiral selector is added in the background electrolyte. Among the great number of selectors reported, cyclodextrins (CDs) are the most widely employed. Neutral and charged CD derivatives with various functional groups can induce different strereoselective interactions [4].

Several pharmaceutical examples have been described in the literature for the analysis of different drugs, by-products and metabolites in different matrices [5]. In order to separate neutral and charged molecules of different size and nature, capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), microemulsion electrokinetic chromatography (MEEKC), capillary isoelectric focussing (CIEF) and capillary gel electrophoresis (CGE) have been developed. Furthermore, the use of non-aqueous solvents such as acetonitrile and methanol can also modify the electrophoretic behaviour of some analytes and present different selectivity in comparison with aqueous buffer solutions. It is noteworthy that generated currents are low in acetonitrile and methanol for performing very rapid separation without an important Joule effect. These methods are well described and can be carried out on commercially available instrumentation with capillaries of 10- to 100-μm internal diameter of different lengths (20–100 cm).

High voltages can be applied (up to 30 kV) thereby allowing very rapid and efficient separations even for stereoselective analysis as reported in Fig. 1. The detection is generally performed by UV-VIS spectrophotometry directly on the capillary. However, even if this detector presents advantages in terms of simplicity, large domain of applications and cost, it suffers from a lack of sensitivity due to the short optical path length afforded by the small internal diameter of the capillary. Sensitivity in the order of μg mL^{-1} is generally obtained for compounds possessing chromophore groups. This sensitivity is sufficient for different applications (e.g. quality control and toxicological studies) but is not adapted to diagnostic purposes, therapeutic drug monitoring or other dosages at low ng mL^{-1} level or to analytes which do not possess chromophore groups (e.g. sugars and peptides).

Different strategies have been reported in the literature in order to improve detection sensitivity, such as the use of laser-induced fluorescence (LIF), mass spectrometry detection (MSD) and electrochemical detection (ECD). LIF permits one to attain low sensitivity at the ppb level but often requires a derivatisation step, since very few compounds are naturally fluorescent. The on-line coupling of CE with mass spectrometry (MS) is a promising combination of two analytical techniques. Among the available ionisation techniques, electrospray

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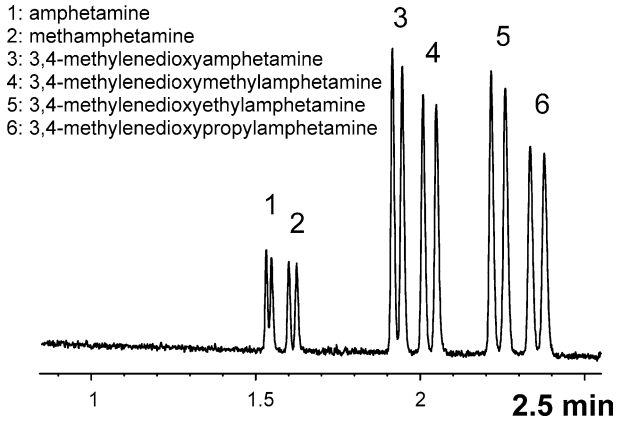

Fig. 1 Rapid chiral analysis of six amphetamine derivatives: fused silica capillary of 50-μm i.d.×32.5-cm total length; 118 mM Tris-phosphate buffer set at pH 3.5; 16 mM hydroxypropyl-β-cyclodextrin; temperature 28°C; voltage 25 kV; UV-detection at 200 nm

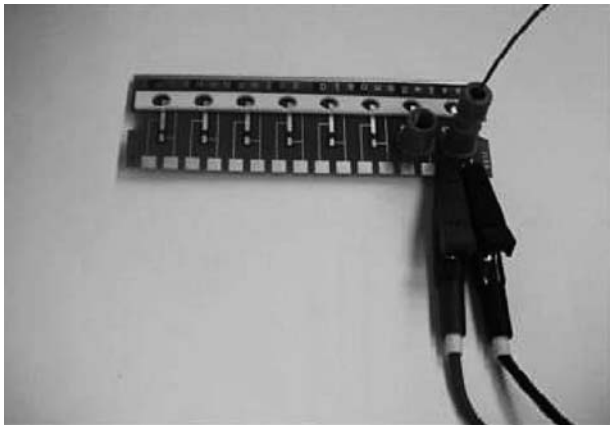

Fig. 2 Representation of a series of eight home-made electrochemical detectors which can be coupled with conventional CE system

ionisation (ESI) is most widely used for on-line CE-MS coupling. By virtue of its high sensitivity and selectivity, MS is a detector of choice in CE. This detector provides information about the mass and potentially the structure of the separated compounds. In this context, the on-line coupling of CE-ESI-MS has evolved into an efficient, sensitive and selective technique for the analysis of drugs and related metabolites [6, 7] as well as for the comprehensive and quantitative metabolome analysis [8].

Finally, ECD has also received great interest owing to its high selectivity and sensitivity for electroactive species such as neurotransmitters and phenolic compounds [9]. It should be noted that a widespread introduction of CE-ECD has been limited by handling difficulties inherent to the classical electrode alignment procedure at the end of the separation capillary and by the difficulty in isolating both separation and detection fields.

New and future trends of CE will be dedicated to miniaturisation. CE is well adapted to miniaturization as demonstrated by Widmer and co-workers a decade ago with the concept of the micro total analysis system (μ-TAS) [10]. Several developments of micro-CE systems have been published recently in the literature especially dedicated to genomics and proteomics, and instrumentation is now commercially available; however, the technique remains challenging and efforts have to be made to obtain robust and sensitive tools. The sample introduction and detection remain challenging tasks for microfluidic systems, and different strategies have to be developed to achieve automated continuous introduction of real-world samples [11]. Most pharmaceutical and biomedical applications need a sensitive and selective analytical method such as the analysis of biochemical markers of cholinergic activity in samples of very limited size (e.g. post-mortem tissue and cerebrospinal fluid) in Alzheimer's disease (AD) and of dopaminergic activity in Parkinson's disease (PD). As already described, CE and more particularly chip-CE coupled with MS or electrochemical detector offer great promise for this purpose. Different laboratories have already demonstrated the great potential of chip-CE with electrospray mass spectrometric detection, and a rugged interface was successfully applied for the quantitative determination of small-molecule compounds isolated from biological samples [12].

Owing to its high sensitivity, low cost and easy miniaturisation, ECD is certainly a detector of choice for conventional (Fig. 2) and microchip CE for several reasons. Microfabrication techniques can help in the alignment of the detector at the end of the capillary. It is possible to use multiple electrodes to increase both selectivity and sensitivity. Furthermore, reduction in the size of the electrode generally increases the detector performance by increasing the current density at the electrode surface and by reducing the capacitive current. Moreover, the decrease in detection volume into the sub-nanolitre range does not dramatically reduce the performance of the electrochemical detector [13, 14]. Owing to its fast response time, ECD is fully compatible with rapid separation, and a larger working potential range can be applied with non-aqueous solvent. Last but not least, plastic chip-CE-ECD can be used as a disposable analytical tool in biomedical and pharmaceutical fields for electroactive drugs. Finally, it should be noted that contactless conductivity detection has recently been introduced in CE: this configuration was reported in capillary and microchip electrophoresis [15].

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